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Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties

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Many of nature's most striking animal colours are iridescent, exhibiting a high degree of spectral purity and strong angular dependence of intensity and hue. Although a growing number of studies have detailed the intricate mechanisms responsible for producing iridescent colours, few attempts have been made to describe their dynamic appearance in ecologically and behaviourally realistic contexts. We suggest that the optical properties unique to iridescent structural colours are important for understanding how they function as signals during behavioural interactions. Using males of the orange sulphur butterfly, *Colias eurytheme*, which exhibit an iridescent ultraviolet (UV) reflectance on their dorsal wing surfaces, we develop a holistic framework for inferring the appearance of this signal to conspecifics under field conditions that incorporate data on their spectral sensitivity. We show that, during flight, the UV signal is brightest within a wing beat cycle when viewed from directly above the male. Spectral properties of the signal under natural lighting indicate that male wing colour should be readily perceived and distinguished from that of females and from the dark green visual background of UV-absorbing vegetation. Finally, our analyses permit predictions regarding how signal senders and receivers should orientate themselves for maximal transmission and reception of this ultraviolet iridescent signal.

ADDITIONAL KEYWORDS: colour signal – iridescence – Lepidoptera – Pieridae.

INTRODUCTION

Animals produce colour signals in an astonishing variety of ways, using pigments, nanostructures, and bioluminescence (Bradbury & Vehrencamp, 1998). Some of the most intriguing colour signals are 'iridescent', namely those having hues and intensities that change with receiver position relative to the light source and sender (Land, 1972). Although iridescent colours are often brilliant, the restricted conditions under which they can be viewed pose special challenges for communication. Poulton (1890) was the first to suggest that senders and receivers of iridescent signals should position themselves relative to each other and the light source such that signal transmission is maximized. A qualitative test of this idea by Hamilton (1965) showed that male Anna's hummingbirds (*Calypte anna*) orientate their courtship flights relative to the sun so that the iridescent reflection of the gorget is directed toward the female. This is the only study of which we are aware that explicitly addresses the challenges of iridescent signals under natural conditions. Clearly, more studies are needed to develop our understanding of how iridescent signals are used in realistic behavioural and ecological contexts. This is the goal of the present study.

Iridescent colours are produced by structural means, as has been shown for a variety of invertebrates (Parker, 1995, 1998; Vulinec, 1997; Parker, McKenzie & Ahyong, 1998; Chae & Nishida, 1999; Schultz, 2001; Vukusic, Wooton & Sambles, 2004) and vertebrates (Bleiweiss, 1985, 1992a, b; Lythgoe & Shand, 1989; Nagaishi & Oshima, 1992; Evans, 2003; Brink & van der Berg, 2004; McGraw, 2004; Hill, Doucet & Buch-

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holz, 2005). Especially well known are the mechanisms that produce iridescent colours in the Lepidoptera (butterflies and moths) (Brink & Lee, 1999; Tada *et al*., 1999; for reviews, see also Ghiradella, 1991; Kemp, 2002; Vukusic & Sambles, 2003).

In the sulphur butterflies (Family Pieridae, Subfamily Coliadinae), modified scales may be present on the dorsal wing surfaces that produce a brilliant iridescence in the ultraviolet (UV) wavelength (Ghiradella *et al*., 1972; Ghiradella, 1974, 1989; Ghiradella & Radigan, 1976; Rutowski *et al*., 2005); these scales are very similar in structure to those that produce the blue iridescence of *Morpho* butterflies (Kinoshita & Yoshioka, 2005). In coliadines, it is the males that exhibit the brightest UV iridescence, whereas females either lack or have greatly reduced UV patterning (Brunton & Majerus, 1995; Kemp, Rutowski & Mendoza, 2005). Male-limited UV reflection (Fig. 1) in the orange sulphur butterfly (*Colias eurytheme* Boisduval) serves as an important signal in both intra- and intersexual interactions. Males are repelled by UV reflectance, but females are more likely to mate with males that have bright UV reflectance (Silberglied & Taylor, 1978; Rutowski, 1985; Papke, Kemp & Rutowski, in press). Nevertheless, few efforts have been made to evaluate quantitatively how this signal may appear to conspecifics in nature (Rutowski, 1977).

The aim of the present study is to describe the salient features of this signal as it appears to conspecifics during aerial interactions when visual assessments are likely to be made. Butterflies offer a special opportunity for detailing the properties of an iridescent signal for a number of reasons. Aside from some relatively minor flexing of butterfly wings during flight (Steppan, 1996), the wing surfaces bearing the reflecting structures (scales) are planar rather than curved (in contrast, for example, to a bird's body or individual feather; Osorio & Ham, 2002). In addition, the wings on which the reflecting scales reside are moved in a stereotyped and symmetrical up-and-down motion in flight. These features simplify the task of describing the signal's properties and potential appearance to conspecifics.

Several complementary techniques are required to describe sufficiently the appearance of the *C. eurytheme* male iridescent signal under natural conditions. We use UV video imaging (Eisner *et al*., 1969) to determine how the relative positions of the sun, sender, and receiver affect the intensity of the UV signal as a male's wings move in flight. These data are coupled with data on wing beat rates to infer the temporal structure of the signal. To estimate conspecific perception of the UV signal under field conditions, we incorporate *C. eurytheme* spectral sensitivity (Post & Goldsmith, 1969) and the spectrum of ambient illumination into our analysis of male wing coloration. The perception of male wing coloration then is contrasted with female wing coloration and with the visual background against which the wings are viewed. Finally, we discuss the implications of these analyses for understanding the behavioural biology of these butterflies as well as other organisms that possess iridescent colour signals.

MATERIAL AND METHODS

Animals

The butterflies used in this study came from alfalfa fields in the vicinity of Chandler, Arizona. Specimens either were collected in the field or reared in the laboratory from eggs laid by field-caught females, as described previously (Rutowski, 1985).

Environmental context

As a group, sulphur butterflies favour open field environments (Scott, 1986), which has implications for the rationale and procedures for this study. In open environments, the most significant source of ambient illumination is radiation coming directly from the sun. Although the solar orb subtends a solid angle of only 0.5° in the sky, direct radiation from this source is many orders of magnitude more intense than the total irradiation coming from all other points in the sky (Endler, 1990). We therefore use the properties of direct solar radiation to evaluate how wing surfaces will appear to conspecifics in the field. Likewise, when studying the signal's appearance and properties in the laboratory, we used a point source of light to simulate natural illumination. Given that male UV iridescence is restricted to the dorsal wing surfaces, and that our study species occurs predominantly in agricultural alfalfa fields, we use the spectral properties of alfalfa to characterize the visual background against which the iridescent signal is viewed.

Spatial properties

We describe light source and receiver positions relative to the horizontal plane surrounding a butterfly in flight and relative to the anterior–posterior axis of the body. We use 'elevation' and 'azimuth' as defined in Table 1. For example, a receiver at elevation 45° and azimuth 90° would be located at 45° above the horizon and perpendicular to the long axis of the subject.

To assess qualitatively the male's UV signal, we used digital imaging techniques. Video and still digital cameras were fitted with a Tiffen 18A filter that absorbs wavelengths in the range of 400–700 nm. We illuminated specimens with light from a tungsten–halogen fibre optic source that had been passed through an infrared-blocking filter. This combination of filters and light source allowed only UV light

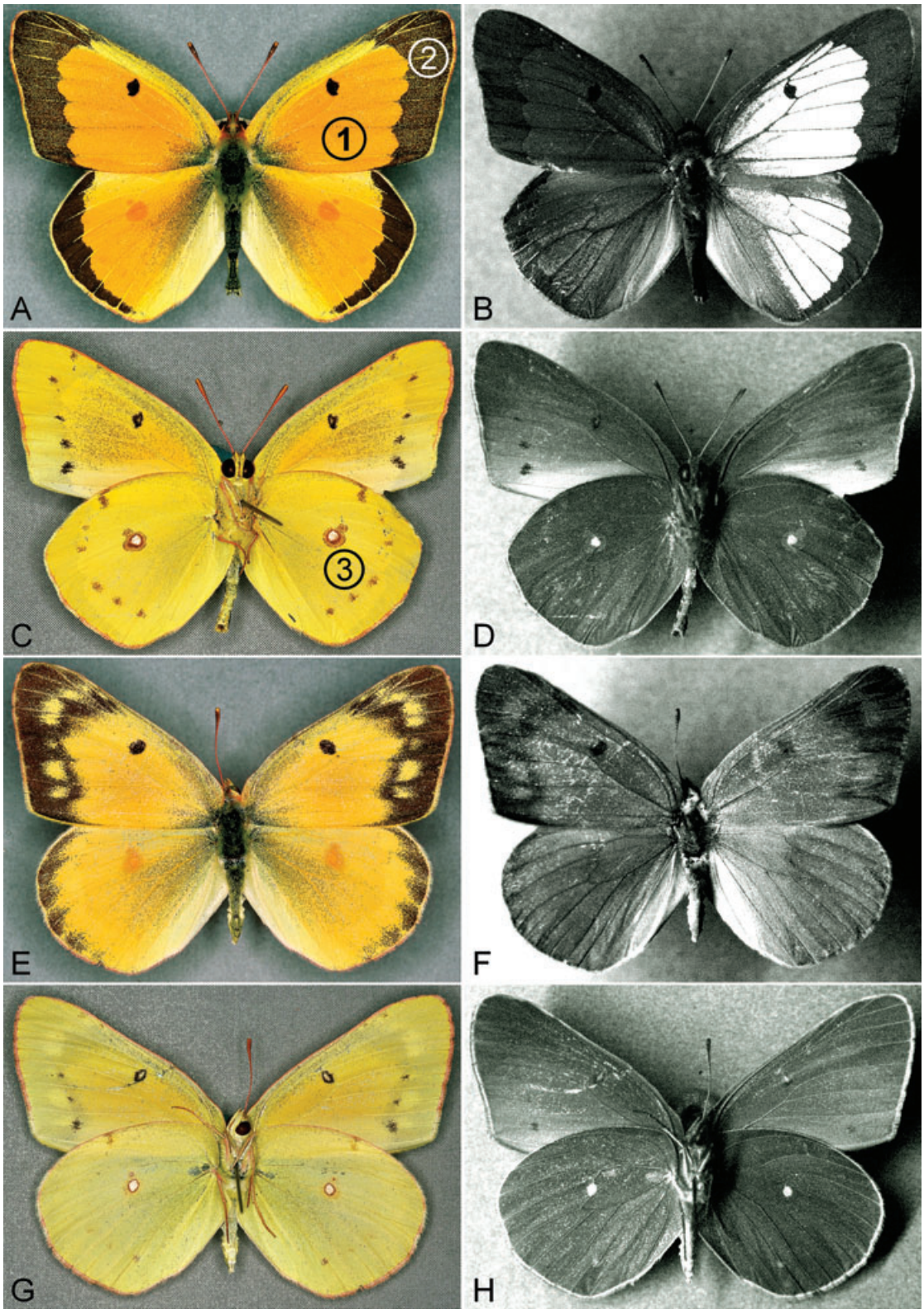

Figure 1. Dorsal and ventral wing surfaces of male and female *Colias eurytheme* in visible (left panels) and ultraviolet (right panels) light. A, B, male dorsal surface. C, D, male ventral surface. E, F, female dorsal surface. G, H, female ventral surface. In (A) and (C), numbers in circles refer to spectral measurement locations for all specimens: (1) dorsal forewing central orange area, (2) melanic margin, and (3) ventral hind wing.

Table 1. Terms, abbreviations, and symbols used in the present study

Term and/or symbol	Definition
Elevation	The angular bearing in the vertical plane (i.e. pitch) of the light source or perceiver above the horizontal plane.
Azimuth	The angular bearing in the horizontal plane (i.e. yaw) to the point directly below the light source or receiver
Angular span	The angle, in degrees, through which the wing passes when the UV iridescence is visible during a down stroke
Spectrum	Amplitude per wavelength distribution of light
Brightness	Perceived intensity
Hue, *H*	Vernacular for 'colour', e.g. red, blue; determined by spectral curve shape and wavelength of peak intensity
Hue angle	Circular coordinate of colour score in colour space
Chroma, *C*	Measure of colour saturation determined by steepness and sign of slope between lowest intensity and highest intensity portions of the spectrum
Reflectance, *R*	Spectrum returned from an object illuminated with 'white' light (i.e. illuminant with equal emission intensity at all wavelengths of interest)
Radiance	Spectrum of light returned from an object illuminated with a biased (nonflat) emission spectrum
Perceived radiance	Radiance multiplied by a species' normalized spectral sensitivity function
Quantum catch, Q_C	Quanta of photons absorbed by photoreceptors considered for a specified set of wavelengths
λ_{max}	Wavelength of maximum intensity
Colour contrast, *CC*	Euclidian distance between two points in colour space
UV+	Wing orientation relative to collector in which UV reflectance is maximized
UV–	Wing orientation relative to collector in which UV reflectance is minimized, defined here as orientation to collector turned 180° on a flat surface from UV+ position

UV, ultraviolet.

(λ_{max} = 380 nm, range = 350–400 nm) to be received by the cameras.

Observations were made on dead specimens, spread in standard entomological style and pinned to a universal stage that allowed measured rotations of the specimen around the long axis of the body. For a given light source and camera position, we rotated the specimen in this way to simulate, albeit in very slow motion, the movement of the wings during flight. Specifically, the specimen was positioned with the right pair of wings orientated vertically with wing tips upward (90°). The wings then were moved as if in a downstroke until the wing tips were orientated directly downward (–90°). During this process, the appearance of the right wing pair was observed through the UV imaging system. We noted the wing angles between 90° and –90° during the down stroke at which: (1) the right forewing first reflected UV from the entire distribution of UV reflecting scales and (2) the UV reflection first began to disappear from parts of the right forewing. This procedure was carried out independently for the right hind wing, and again for each member of the left wing pair.

Observations with the UV imaging system were made for three male *C. eurytheme* specimens from 11 receiver positions for each of four light source positions. All light source positions had an elevation either of 45° or 90°, which are typical of solar elevations that occur during the time of day when these butterflies are flight active.

From these data, we extracted two variables to characterize the signal. First, for each individual, we visually determined the number of wings that simultaneously reflected UV maximally during the down stroke. This number served as a simple index of signal brightness. The data for each light source position were plotted on spheres that depict viewer positions around a flying male; isoclines were visually interpolated from the plotted data. These isoclines demarcate regions of similar signal brightness. Second, for the right forewing of each specimen, we measured, in degrees, the range of wing sweep over which UV was reflected under each set of light and camera positions. This provided a measure of the 'angular span' of UV reflectance during a down stroke.

Temporal properties

We collected data on wing beat rate and wing movements in the following manner. The ventral thorax of

a live field-caught male was glued to the end of a wooden applicator stick (diameter 3 mm). By blowing on the front of the male's head, we elicited sustained flight-like wing beats. This flight-like motion was recorded at 250 frames s^{-1} from directly in front of the male using a high-speed digital video camera (The MotionMeter, Redlake MASD, Inc.). A frame grabber (muTech MV510) captured and output frames as individual TIFF files. The TIFF files were compiled into sequences and were analysed using Image J (available at http://rsb.info.nih.gov/ij/). Frame-by-frame analysis of these sequences allowed us to calculate wing beat frequencies and wing angular velocities. Pairing these data with our data on UV angular duration allowed us to infer the temporal structure of the UV signal during flight.

Spectral properties

Reflectance

For each individual, reflectance spectra were collected from three wing regions (Fig. 1). These regions were the central (orange) area and melanic margins of the dorsal forewing, and central area of the ventral hind wing. The latter was selected because the ventral hind wings are the predominant visible wing area when the butterflies are at rest. Sample size was the same for all wing regions ($N = 35$), except for the male ventral hind wing ($N = 26$).

Reflectance spectra of specimens were obtained as follows. Wings were carefully cut from bodies of dead specimens and mounted on black matte card stock. A mounted wing was placed on a universal stage in a dark room and illuminated with a light beam normal to the wing surface. This light beam was generated by a pulsed-xenon light source (Ocean Optics PX-2) and delivered via an optical fibre (Ocean Optics, diameter 400 μm). The collecting fibre was fitted with a collimating lens (Ocean Optics 74-UV) and positioned at an azimuth of 90° and an elevation of 45° above the wing surface. The light beam entered an Ocean Optics USB2000 spectrometer connected to a desktop computer running OOIBASE32 software. A glass microscope slide coated with magnesium oxide was used as the reflectance standard. Reflectance of this matte white standard was nearly 100% from 300–700 nm, relative to a commercially produced white reflectance standard. A diffuse standard is appropriate for our spectral measurements of UV iridescence because, although being specular, the intensity of the iridescence falls within the reflectance range of our standard (i.e. often less than 100%).

Measuring UV iridescence from the central dorsal wing surface of males required additional procedures unnecessary for other wing areas. Two spectra were obtained from this wing region: one using a spatial arrangement in which UV intensity was maximized (UV+ position) and another in which UV reflectance was minimized (UV–). To obtain the UV+ spectrum, the left forewing was placed on the universal stage with the wing base (i.e. the point of attachment to the body) nearest the collector, which corresponds to a collector position of azimuth 90° and elevation 45° relative to an intact butterfly. Slight adjustments then were made in the position of the wing (with all axes set to 0°) on the stage until the location of the strongest UV reflectance was found. Because minor adjustments in wing angle (rotated around the anterio–posterior axis of the wing) could intensify UV reflectance, wing angle was varied until UV intensity was maximized, and this spectrum was recorded. Next, spectra were obtained incrementally as the wing was moved in 5° steps, to assess reflectance intensity changes with the down stroke of a wing beat. Finally, to obtain a spectrum of central dorsal wing coloration that did not include the contribution of UV iridescence (i.e. UV– orientation), the wing was rotated around its dorso–ventral axis 180° from the UV+ orientation and this spectrum was recorded. The female dorsal forewing lacks iridescence, as do all other wing areas measured in both sexes. For these measurements, no changes in wing orientation on the stage were made, and all stage axes remained at 0°.

Natural ambient illumination

For reasons detailed in the Material and Methods section (Environmental context), direct solar radiation was measured around midday in summer under a clear and cloudless sky, for use in our spectral analyses. An optical fibre fitted with a collimating lens (approximately 3° acceptance angle) was pointed near the sun's corona, taking care to avoid signal saturation. The response of the USB2000 was calibrated with an Ocean Optics LS1-CAL lamp immediately prior to obtaining the solar radiation sample. The resulting spectrum was transformed to photon flux (μmol m^{-2} s^{-1} sr^{-1} nm^{-1}; Endler, 1990) and normalized to an amplitude peak equal to 1 (Fig. 2). The resulting curve corresponds well to the CIE standard illuminant B for direct sunlight (colour temperature 4874 k; http://home.hetnet.nl/~paul-schils/07.01.html), which is a representation of the spectral characteristics of full sunlight.

Perceived radiance

We combined measures of wing reflectance, direct solar radiance, and *C. eurytheme* spectral sensitivity to infer this species' perception of its wing coloration, as well as the contrast of that coloration against a visual background of alfalfa. Colour perception of *C. eurytheme* was estimated by calculating the quantum catch of the combined photoreceptors, Q_c, as:

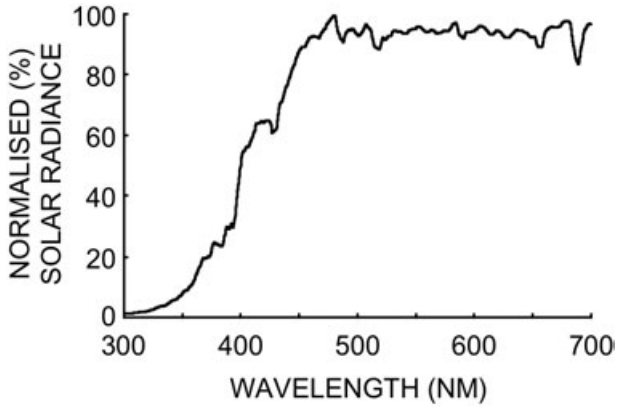

Figure 2. Solar radiance (normalized to wavelength of peak intensity) in a clear, central Arizona sky.

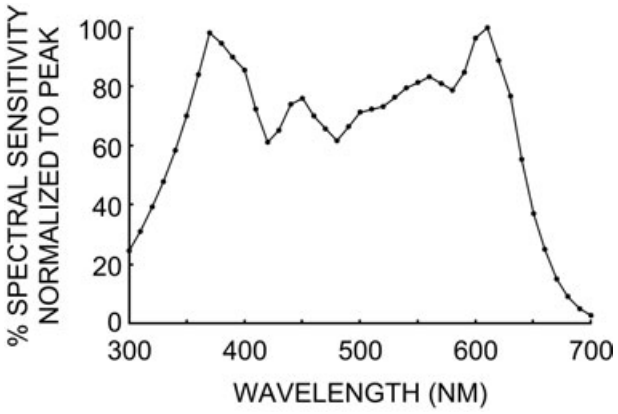

Figure 3. Electroretinograph of *Colias eurytheme* used to approximate spectral sensitivity in this study. Data summarized from Post & Goldsmith (1969).

$$Q_c = \int_{\lambda 300}^{\lambda 700} R(\lambda) SR(\lambda) SS(\lambda) d\lambda \quad (1)$$

where $R\ (\lambda)$ is the reflectance spectrum of the object viewed, $SR\ (\lambda)$ is the normalized solar radiance spectrum illuminating that object, and $SS\ (\lambda)$ is normalized spectral sensitivity integrated over the wavelength range of interest (300–700 nm). Data on *C. eurytheme* spectral sensitivity were obtained from electroretinograms (ERG) of three individuals (gender not reported) in Post & Goldsmith (1969). We extracted wavelength-specific relative sensitivity values from these ERGs and calculated a median value at 10-nm intervals from 300–700 nm (Fig. 3).

We produced 'perceived radiance' spectra for various wing areas and the visual background (alfalfa) by calculating the product of reflectance, normalized solar radiance, and normalized spectral sensitivity at each 10-nm interval from 300–700 nm. These spectra were used in our quantitative analyses of coloration and contrast.

Summarizing coloration

To visualize the distributions of wing colour measures and to facilitate calculation of colour contrasts, each perceived radiance spectrum was reduced to a single point in two-dimensional 'colour space' using the segment classification method of Endler (1990). Spectra were partitioned into four, 100-nm wide colour segments approximately corresponding to UV to violet (300–400 nm; 'U' wavelengths segment), violet to green (400–500 nm; 'S' or short wavelengths segment), green to orange (500–600; 'M' or medium wavelengths segment), and orange to red (600–700 nm; 'L' or long wavelengths segment). The sum (intensity) of each segment (Q_U, Q_S, Q_M, Q_L) then was divided by the entire spectrum's sum from 300–700 nm (Q_T). This calculation eliminates intensity differences among the spectral segments by transforming each segment to a proportion. Subtraction of Q_U from Q_M and Q_S from Q_L produces two values, X and Y, respectively, that are plotted as a single colour score in colour space.

Chroma (C) increases as the Euclidian distance from the colour space origin (i.e. zero on X and Y axes), and was calculated as modified from Endler (1990):

$$C = \sqrt{(Q_L - Q_S)^2 + (Q_M - Q_U)^2} \quad (2)$$

Hue (H) is depicted as the angle of a colour score relative to the top (0°) of the graph's vertical axis, and was calculated as modified from Endler (1990):

$$H = ArcSin(X)/C \quad (3)$$

where X is the x-axis location of the colour score in colour space. Differences in colour contrast (CC) between pairs of wing measures (e.g. contrast with alfalfa of male UV+ dorsal forewing orientation vs. contrast with alfalfa of the UV– orientation) were calculated as the Euclidian distance between pairs of colour scores using:

$$CC = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2} \quad (4)$$

Statistical analysis of wing spectra

Prior to statistical comparisons, all data distributions were examined, and in no case was a distribution found to deviate significantly from normality (Kolmogorov–Smirnoff one-sample test, for all tests $P > 0.05$). In comparing intensity differences between the UV+ and UV– orientations of the male dorsal forewing, total intensity was calculated for the entire butterfly visible spectrum (300–700 nm) as well as for each 100 nm-wide spectral segment. A two-way analysis of variance (ANOVA) was used to test for main effects of wing orientation and spectral segment. For significant

effects, paired *t*-tests were used to test for differences between paired UV+ and UV– samples taken from the same specimens, and independent *t*-tests were used to test for differences between sexes (see Results). Levene's adjustment for unequal variances was used to determine degrees of freedom in independent *t*-tests. *P*-values in multiple pairwise tests were adjusted using the sequential Bonferonni method (Rice, 1989). Sex differences in hue, chroma, and colour contrast with the visual background (Euclidian distance in colour space) were tested in a manner similar to that of spectral intensity, except that separate tests for 100-nm wide spectral segments were not conducted. All statistical tests were carried out with SPSS for Windows (version 10.1).

RESULTS

Spatial properties

The magnitude of male UV reflectance changed dramatically with changes in wing angle relative to the horizontal plane during a down stroke. With illumination normal to the wing surface and the video camera at azimuth 90° and elevation 45°, UV reflectance began to appear just after the wing passed through horizontal (Fig. 4). With increasing depression of the wing, intensity and area of UV reflectance increased until reaching maximum amplitude at an angle of approximately –30°. This maximum was maintained from approximately –30° to –45°, after which intensity and area of UV reflectance decreased as the wing continued to be moved downward. By –70°, UV reflectance had all but disappeared in the video image.

Figure 5 summarizes how the maximum number of wings, fully and simultaneously showing UV reflectance during a down stroke, changed with viewer position and light source location. When the light source was normal to the horizontal plane (i.e. above and perpendicular to the butterfly specimen), the maximum number of wings fully displaying UV was greatest when the male was viewed from directly overhead (Fig. 5A). When the light source was moved to a position in front of and above the butterfly (azimuth 0°, elevation 45°), the viewing position that yielded this maximum number was shifted to a position slightly behind the male. When the light source was moved to the right side of the butterfly (azimuth 90°, elevation 45°), the maximum number of wings fully reflecting

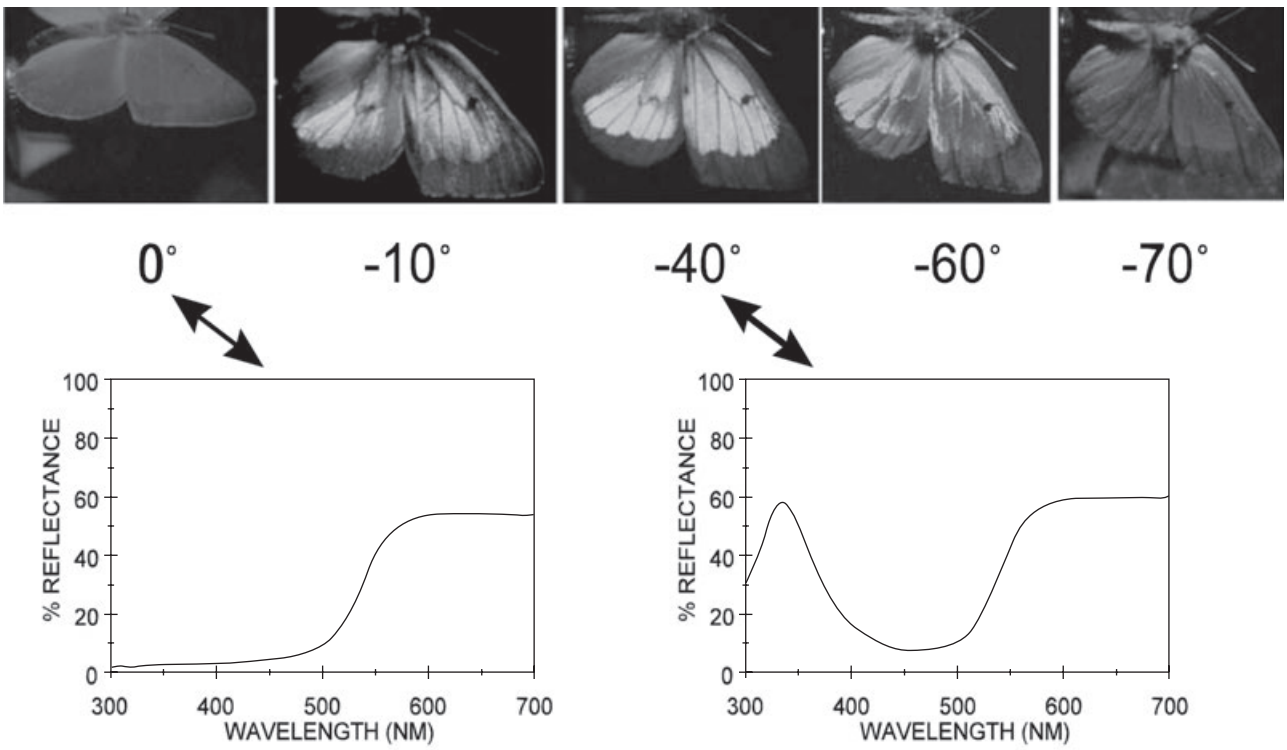

Figure 4. A, changes in appearance in ultraviolet (UV) imaging of male *Colias eurytheme* wings as they are swept through a down stroke of the wing beat cycle. In this example, the light source was located at elevation 90° and azimuth 0°, and the camera was located at elevation 45° and azimuth 90°. B, reflectance spectra of the wings showing the absence (left) and near maximum (right) reflectance of the iridescent structural UV coloration.

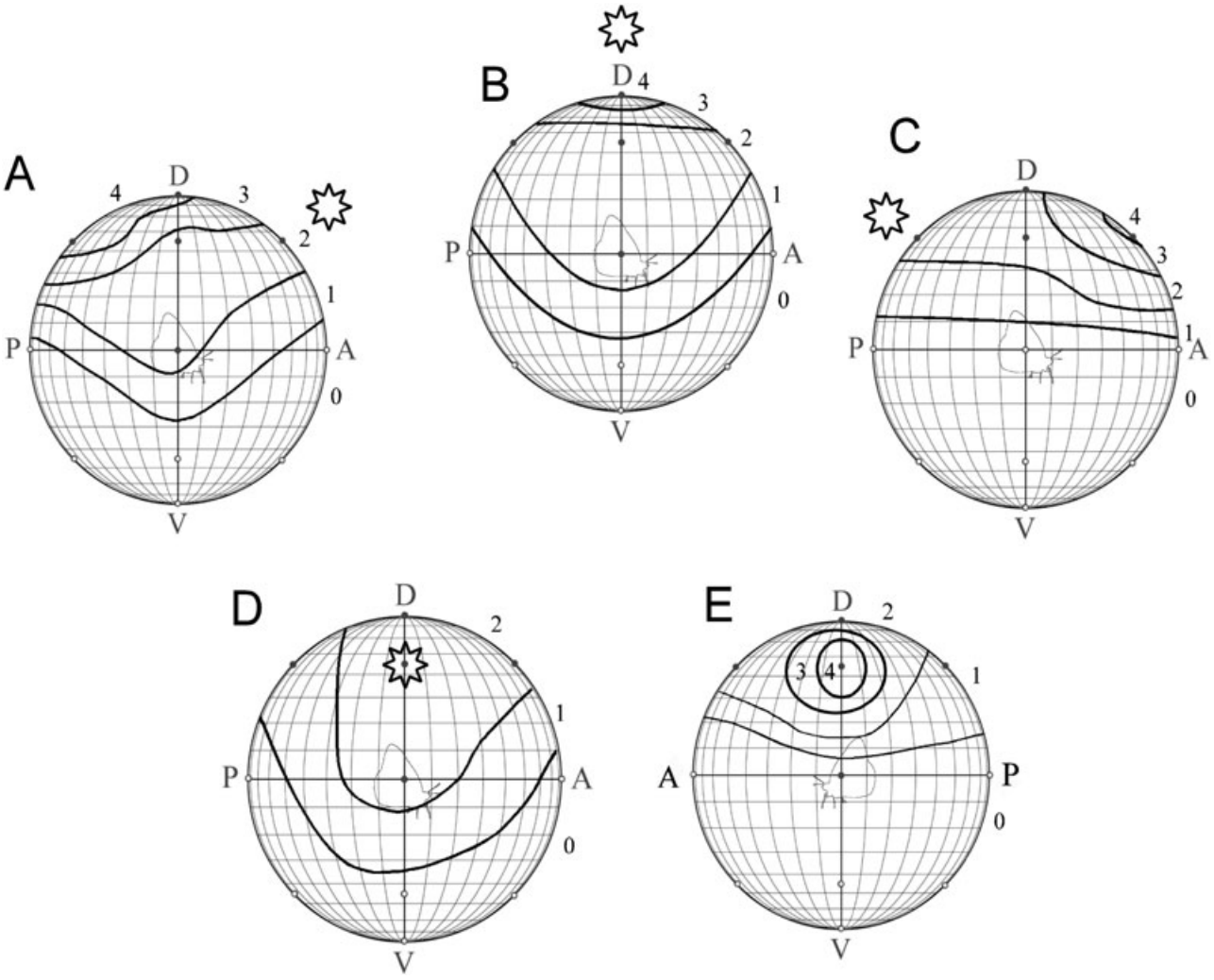

Figure 5. Changes in the number of wings that fully and simultaneously reflected ultraviolet light, as a function of light source location (shown by sunburst icon) and receiver location in the space around a male in flight ($N = 3$). The light source locations examine were: A, elevation 90°; azimuth 0°; B, elevation 45°, azimuth 0°; C, elevation 45°, azimuth 180°; D, elevation 45°, azimuth 90°, sample points from animal's left side; E, elevation 45°, azimuth 90°, sample points from animal's right side (light source location not visible). D, dorsal; V, ventral; A, anterior; P, posterior. Numbers of increasing value correspond to isoclines of increasing brightness (i.e. isocline 4 is the brightest; isocline 0 is the least bright).

UV was observed from the opposite side, but still at elevations around 45°. UV was not visible during a down stroke for viewing positions below and directly in front of or directly behind the male. In summary, the location from which UV reflectance should be perceived as brightest is from positions above a flying male.

Angular span and UV reflection

In three specimens, and over a range of light source and collector positions, we measured the forewing angles during a down stroke for which a full UV reflection was visible. The angular span over which UV reflectance was observed from all wing areas bearing UV reflecting scales was 5.3–24.5°. The only clearly discernable pattern of variation was that, for each illuminant position, the maximum angular span occurred when the collector was positioned at an elevation of approximately 45°.

For a larger number of males, the angular span was measured for only the forewing UV reflection during a down stroke. For these measurements, the light source was at azimuth 0° and elevation 90° and the camera at azimuth 90° and elevation 45°. Under these conditions, the angular span of the forewing flash averaged 13.8° ± 5.36° (range = 3–24°, $N = 50$).

Temporal properties

The restrained males that we filmed moved their wings in ways similar to those observed in normal flight. Nevertheless, we understand that tethered animals may exhibit flight patterns that differ from flight in field conditions, and that, even in the field, flight movements may change rapidly and drastically with changes in behavioural demands. We therefore view the measurements described below as only first approximations of wing movements during flight. We analysed high-speed film footage of two males to characterize wing movements so that we could infer the temporal structure of UV reflectance changes during flight. Duration for full wing beats (contralateral wing tips touch on up and down stroke) was 118 ± 16 ms (range = 96–172 ms; $N = 52$) for one male and 128 ± 8 ms (range = 112–142 ms; $N = 35$) for another. These durations equate to average wing beat rates of 8.5 and 7.8 Hz, respectively. Angular velocity reached a maximum exceeding $5000° s^{-1}$ as the wings passed through the horizontal plane, and a minimum ($0° s^{-1}$) when wings met at the top and bottom of a wing stroke (Fig. 6).

Spectral properties

Changes in reflectance during wing beat

We measured UV reflectance spectrophotometrically to quantify changes in UV intensity during the down stroke of a wing beat. With the illuminating beam normal to the horizontal plane, the collector at azimuth 90° and elevation 45°, and the wing in the UV– position, UV reflectance intensity was only approximately 3% ($\lambda_{max} = 340$ nm; Fig. 7C). By contrast, UV reflectance intensity in the UV+ position was approximately 75% ($\lambda_{max} = 340$ nm) (Fig. 7A). UV peak intensity was greater than reflectance elsewhere in the spectrum (64% at $\lambda_{max} = 620.5$ nm). Maximum UV reflectance was observed between wing angles of –30 and –50° (Fig. 8), reinforcing our findings from the video image analysis.

Changes in perceived brightness, hue, and chroma during wing beat

Incorporating data on *C. eurytheme* spectral sensitivity and local solar radiance in our analyses permitted us to simulate perceived wing brightness and colour. Results of a two-way ANOVA showed a significant main effect on perceived wing brightness of spectral segment ($F_{3,272} = 22344.3$, $P < 0.001$) but not wing orientation ($F_{3,272} = 0.0$, $P = 1.0$). However, a significant wing orientation × segment ($F_{3,272} = 396.2$, $P < 0.001$) interaction was found. This interaction arose from the UV (300–400 nm) and short (400–500 nm) wavelength segments being significantly brighter in the UV+ wing orientation, whereas the middle (500–600 nm) and long (600–700 nm) wavelength segments were significantly brighter in the UV– orientation (Table 2).

The results of perceived radiance calculations also indicated significant differences in hue and chroma between: (1) the two orientations of the male dorsal forewing, (2) the UV– orientation of the male dorsal forewing and the female dorsal forewing, (3) the male and female melanic dorsal forewing border (hue only), and (4) the male and female ventral hind wing (Table 3; Figs 9, 10). Notably, the female ventral hind wing reflected more short wavelengths than the male ventral hind wing (Figs 9D, 10), and exhibited a greenish hue (mean $\lambda_{max} = 553$ nm; Fig. 7H) very much like that of alfalfa leaves (mean $\lambda_{max} = 552$ nm; Fig. 7B).

Segment classification analysis of the perceived radiance spectra permitted the evaluation of colour contrast independent of brightness. Euclidian distance between the male and female forewing in colour space was significantly greater with the male forewing in the UV+ orientation than in the UV– orientation

Table 2. Perceived brightness (300–700 nm) comparisons of wing surfaces in *Colias eurytheme*

Comparison	*t*	*P*	d.f.	Brightness difference
A. ♂ dfw: UV+ vs. UV–	17.2	< 0.001	34	(see below)
By segment:				
300–400 nm	26.0	< 0.001	34	UV+ brighter
400–500 nm	17.1	< 0.001	34	UV+ brighter
500–600 nm	– 21.1	< 0.001	34	UV– brighter
600–700 nm	– 22.2	< 0.001	34	UV– brighter
B. ♂ dfw UV– vs. ♀ dfw	7.9	< 0.001	53.2	Male brighter
C. dfw melanic margin: ♂ vs. ♀	0.3	0.753	68	NS
D. vhw: ♂ vs. ♀	– 1.0	0.313	58	NS

Degrees of freedom (d.f.) in independent *t*-tests (B, C, D) determined with Levene's adjustment for unequal variances. UV, ultraviolet; Dfw, dorsal forewing; vhw, ventral hind wing.

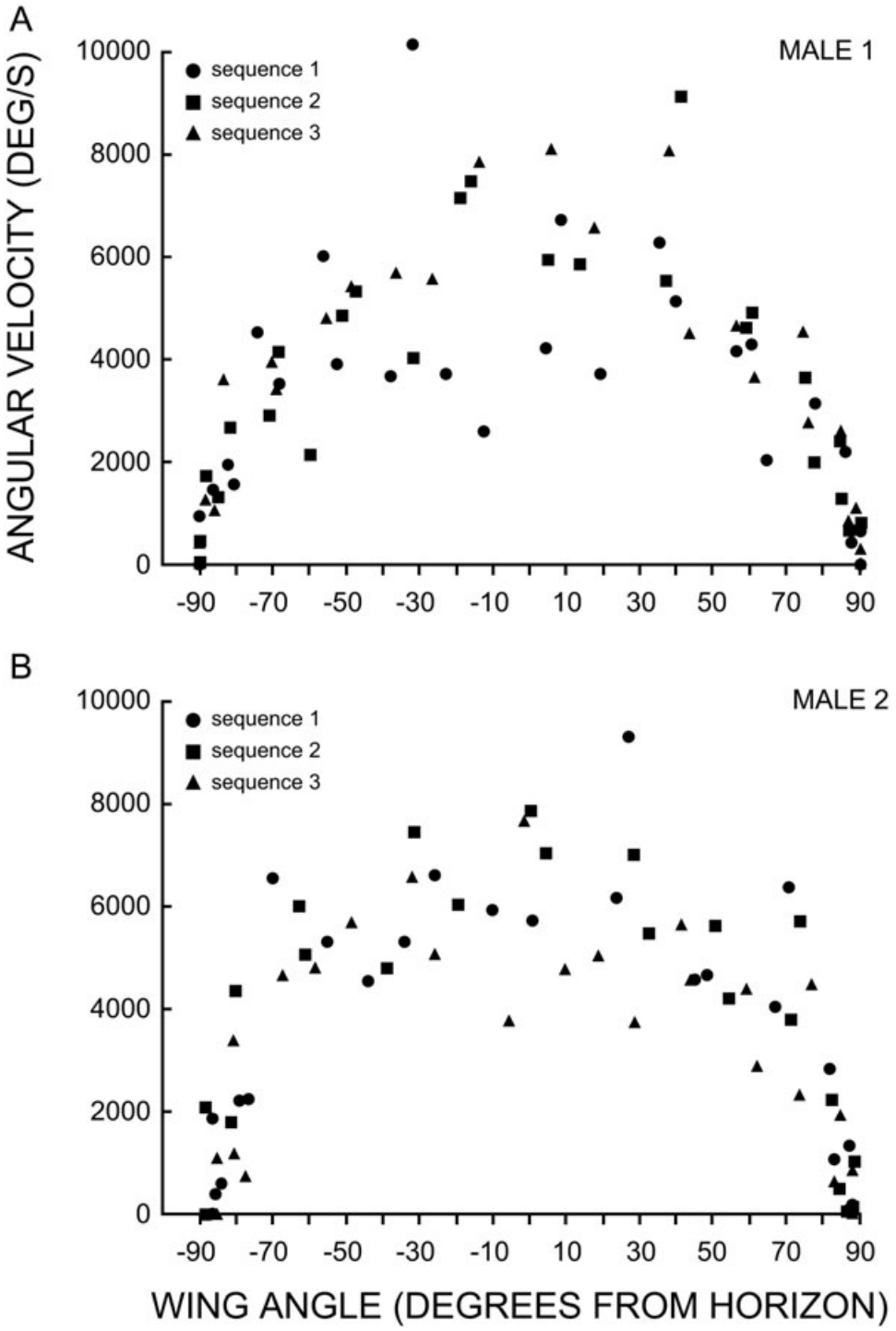

Figure 6. Changes in the angular velocity of the wings during a wing beat. Data are shown for two males and for three full wing beat cycles from each male.

(Table 4, Fig. 10). In other words, male and female dorsal wing surfaces should appear most different in colour when male UV iridescence is maximally visible. The male forewing also was significantly more distant in colour space from alfalfa when in the UV+ orientation than in the UV– orientation. Finally, male hind wing coloration was significantly more distant from alfalfa than was female hind wing coloration (Table 4,

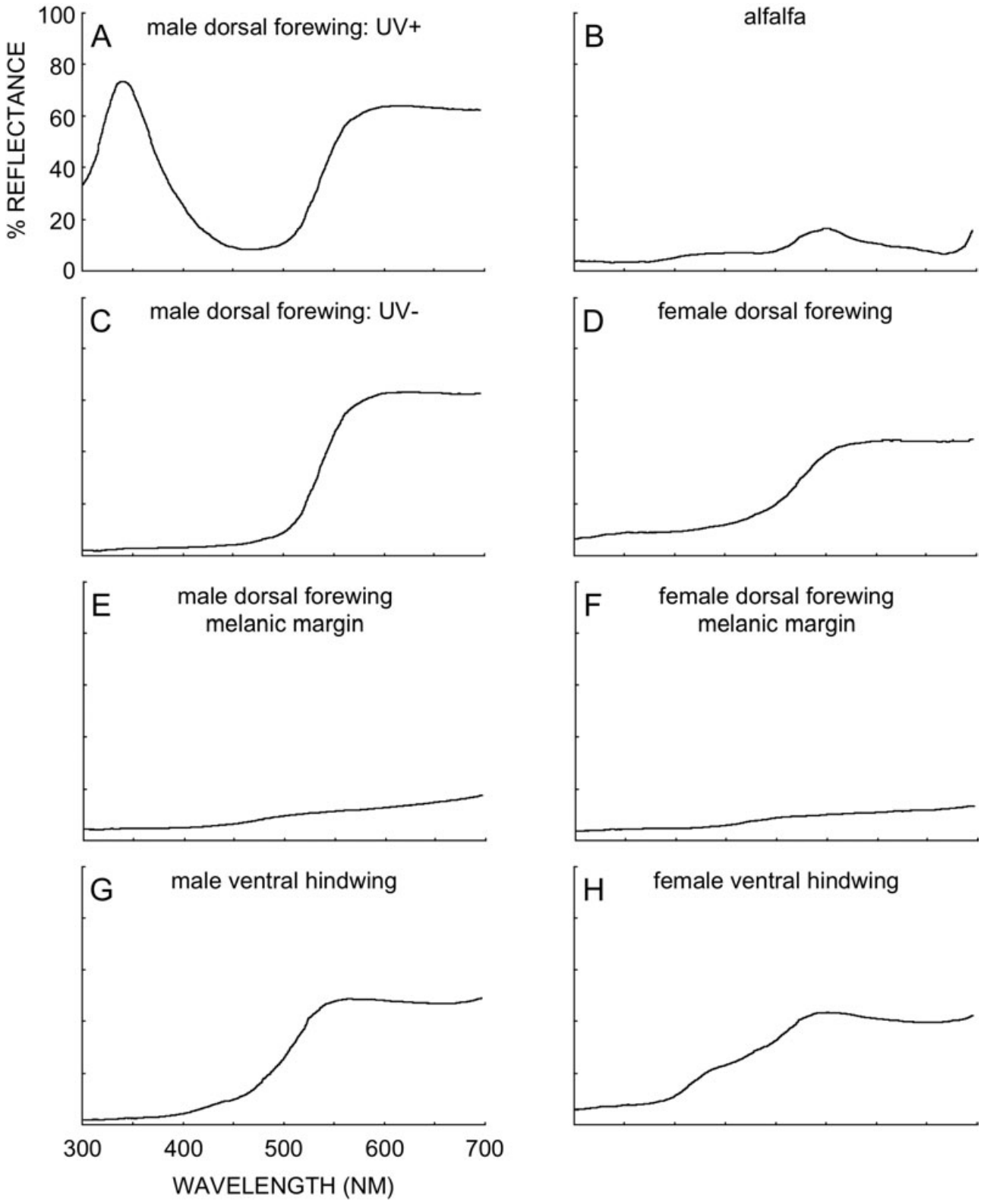

Figure 7. Mean reflectance spectra: A, male dorsal forewing using a spatial arrangement in which ultraviolet (UV) intensity was maximized (UV+); B, alfalfa; C, male dorsal forewing using a spatial arrangement in which UV reflectance was minimized (UV–) orientation; D, female dorsal forewing; E, male melanic margin; F, female melanic margin; G, male ventral hind wing ($N = 26$); H, female ventral hind wing. Sample size for all, except male ventral hind wing, $N = 35$.

Fig. 10), again emphasizing the conspicuousness of male coloration relative to a background of alfalfa compared to that of the female. In summary, these results indicate that males exhibit greater colour contrast against a visual background of alfalfa than do females, regardless of which wing surface or orientation is considered.

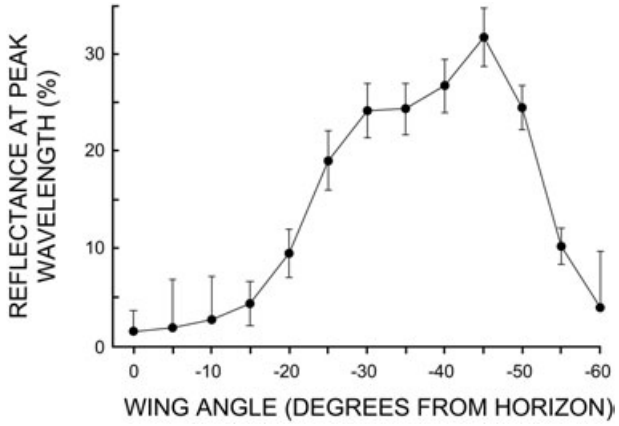

Figure 8. Intensity changes in ultraviolet reflectance peak wavelength (mean ± standard error) with changes in wing angle ($N = 10$).

DISCUSSION

A number of previous studies have documented the characteristics unique to iridescent signals under laboratory conditions, and others have simulated the perception of animal pigmentary colours in realistic settings. However, to our knowledge, this is the first study to estimate quantitatively how an iridescent animal signal is perceived by conspecifics as that signal moves through space and time in a real-world environment. Our results permit several conclusions about the properties of the iridescent UV signal produced by male orange sulphur butterflies.

Spatial and temporal properties of the UV signal

An important conclusion from our spatial analysis is that the perceiver will see the greatest number of wings simultaneously reflecting UV, and therefore the bright-

Table 3. Hue and chroma comparisons of wing perceived radiance (300–700 nm) in *Colias eurytheme*

Comparison	Measure	*t*	*P*	d.f.
A. ♂ dfw: UV+ vs. UV–	Hue	3.0	< 0.005	34
	Chroma	– 24.2	< 0.001	34
B. ♂ dfw UV– vs. ♀ dfw	Hue	– 15.8	< 0.001	47.8
	Chroma	20.7	< 0.001	45.3
C. dfw melanic margin: ♂ vs. ♀	Hue	– 4.4	0.001	67
	Chroma	– 0.4	NS	62.8
D. vhw: ♂ vs. ♀	Hue	– 20.4	< 0.001	56.2
	Chroma	21.3	< 0.001	57

Degrees of freedom (d.f.) in independent *t*-tests determined with Levene's adjustment for unequal variances.
UV, ultraviolet; Dfw, dorsal forewing; vhw, ventral hind wing.

Table 4. Perceived radiance colour contrast (Euclidian distance in colour space) of wing and visual background (alfalfa)

Distances compared	*t*	*P*	d.f.	Difference
A. ♂ UV– dfw to ♀ dfw ♂ UV+ dfw to ♀ dfw	–3.9	< 0.001	68	♂ UV+ > ♂ UV– from ♀ dfw
B. ♂ UV+ dfw to alfalfa ♂ UV– dfw to alfalfa	–6.3	< 0.001	56.2	♂ UV– > ♂ UV+ from alfalfa
C. ♀ UV– dfw to alfalfa ♂ dfw to alfalfa	25.4	< 0.001	60.9	♂ UV– > ♀ dfw from alfalfa
D. ♀ vhw to alfalfa ♂ vhw to alfalfa	15.4	< 0.001	39.1	♂ vhw > ♀ vhw from alfalfa

Degrees of freedom (d.f.) in independent *t*-tests determined with Levene's adjustment for unequal variances.
UV, ultraviolet; Dfw, dorsal forewing; vhw, ventral hind wing.

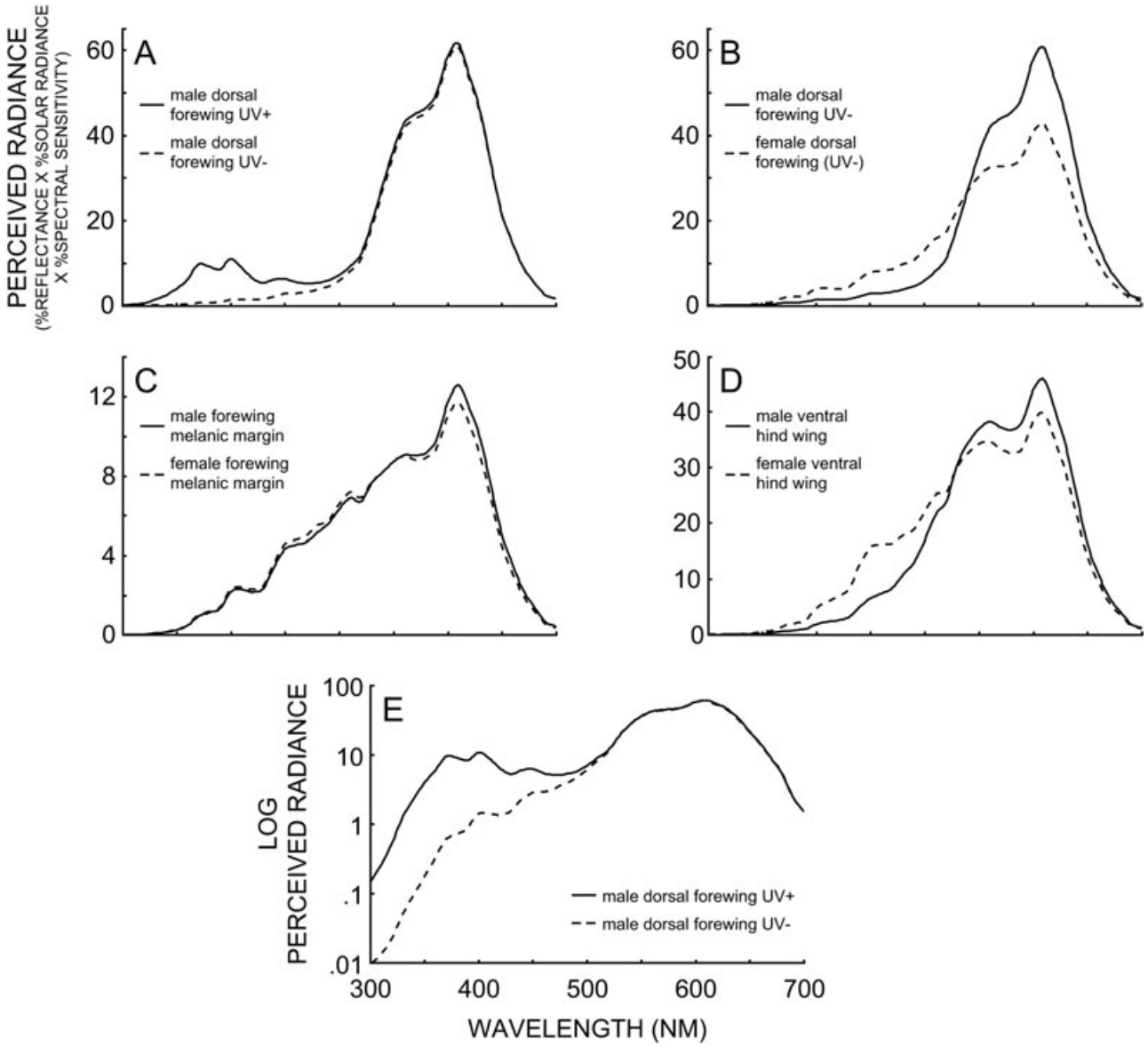

Figure 9. Estimated perceived radiance of wing regions examined in the present study.

est signal, when positioned directly above the male in flight. This will be true for all solar elevations that are typical of the time of day when these animals are active in the field. Research on visual acuity in *C. eurytheme* is consistent with this proposition: although males have greater visual acuity than females throughout most of their visual field, female acuity matches that of males' among ommatidia directed ventrally (Merry *et al*., 2006). It is these ommatidia that would be used to view a male UV signal from above.

One caveat worth mentioning is that our data were obtained with the pitch of the body axis set at 0°, although, during flight, the long axis of the body may often deviate from horizontal. However, the effects of such deviations can be assessed. For example, if we take the arrangement of the sun and butterfly in Figure 5B and incline the anterior end of the butterfly 45° above the horizon, the relationship of the sun to the butterfly will be the same as that depicted in Figure 5A. It can be seen that, even with this 45° incline in body orientation relative to the position of the sun, the UV signal still would appear brightest when viewed from above the male. Thus, our primary conclusions appear to be generally robust, and reinforce our notion that the interactions of males with conspecifics should exhibit a distinctive spatial form. We predict that, in the early stages of interaction, approaching males should position themselves above a conspecific to

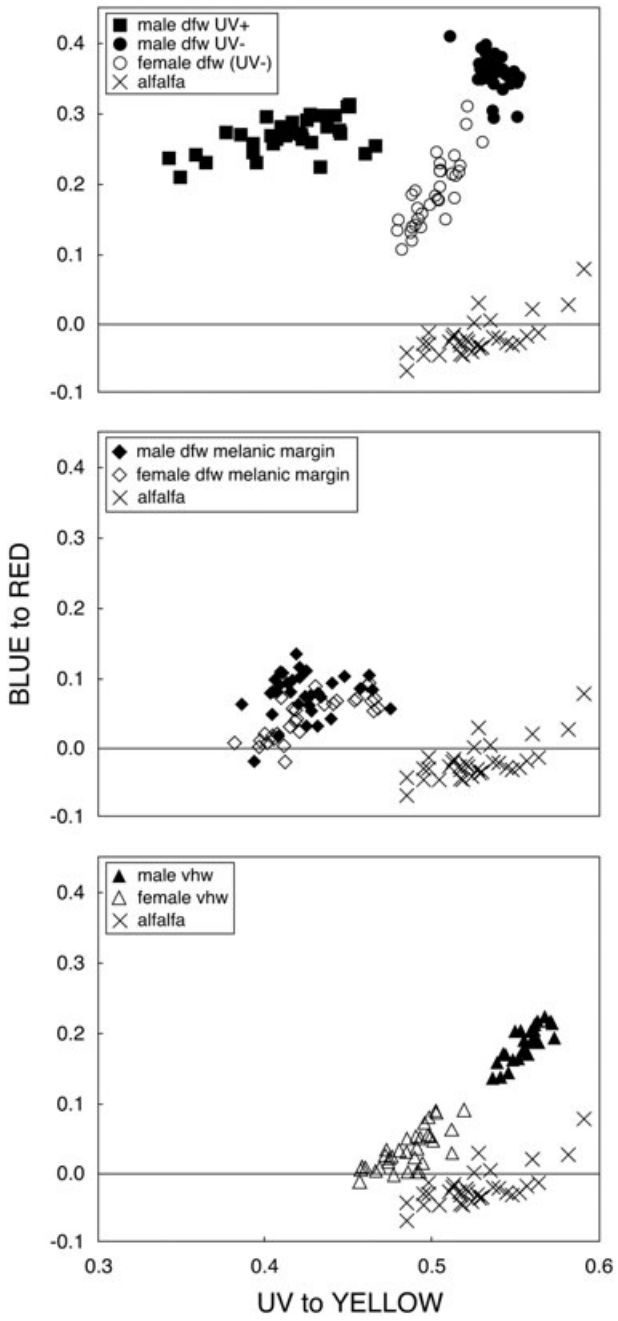

Figure 10. Perceived radiance colour scores plotted in the colour space of Endler (1990). UV, ultraviolet.

assess its sexual identity. If the approaching male does not perceive UV reflectance, then the conspecific is female and the male should position himself below her to maximize her ability to assess his UV signal. Determining whether male behaviour in conspecific interactions is structured in this manner will require further study.

The results of the present study also allow us to define the temporal changes of the UV signal that would occur due to normal movements of the wings in flight, and how these changes might be perceived by a conspecific. More specifically, the UV signal will consist of two amplitude pulses during the wing beat cycle: one on the down stroke and another on the upstroke. Given that mean wing beat frequency was calculated as approximately 8 beats s^{-1}, the UV signal typically should pulse at about 16 Hz. This pulse rate is well within the flicker fusion frequency of butterflies, which may be in excess of 100 Hz (Rutowski, 2003). Hence, we expect the UV component of male wing coloration to be perceived as a series of rapid colour changes that may contribute to the signal's conspicuousness. However, the individual pulses of UV will be brief. The typical sweep of the wings during which the UV reflection was visible was approximately 14°. As the wings passed through the horizontal plane, their angular velocity reached approximately 5000° s^{-1}; at this wing speed, the UV pulse would be visible for less than 3 ms during a wing stroke. Given that UV signal intensity increases gradually as the wings sweep in each direction (Fig. 8), it is likely that the signal will be perceived over a larger angular span than 14°. If so, then our estimate of the time window available for viewing the signal is conservative.

Although information about signaller quality might be encoded in the temporal structure of the UV pulses, this is unlikely. Changes in wing beat rates occur as a male modulates his flight speed and direction during an interaction, which likely would confound a temporally based assessment of quality. Even if wing beat rates remained constant, the rapidly changing spatial relationships between sender, receiver, and sunlight would result in massive perceptual variation in UV pulse rate and duration.

Because these spatial relationships change during an interaction, we do not expect the hue shift that defines iridescence to play a significant role in the signal. To visualize this hue shift requires that the angle of incidence and the angle of viewing relative to the iridescent surface change in precise and reciprocal ways that are not likely to occur as the wing surfaces, and receiver, move during an interaction.

Spectral properties of the UV signal and sexual dichromatism

Given that direct sunlight is weak in UV compared to longer wavelengths (Fig. 2), the intensity changes in the male's UV signal will be much smaller than is suggested by its measured reflectance (Figs 4, 7). However, the perception of light intensity is not likely to change linearly with radiance but with the log of radiance (Hailman, 1977). Hence, small differences in UV radiance may be perceived as considerable differences

in brightness (Fig. 9E), especially when considering the high sensitivity of *C. eurytheme* to UV wavelengths (Fig. 3).

Female wing coloration is not merely male coloration without UV iridescence. In the central orange area of the dorsal wing surface, female radiance is lower in chroma and intensity than male wings that are in the UV– position. Female melanic wing markings differ from those of males in hue (Table 3) as well as in colour pattern (Fig. 1). The ventral wing surfaces of males and females differ in hue and chroma but not in brightness. Silberglied & Taylor (1978) showed that this difference in ventral wing colour was sufficient for sexual discrimination by males.

Sexual dichromatism in ventral wing coloration raises the question of why it is present, given that male UV iridescence is more than adequate for sex discrimination. We speculate that males may benefit from sex-specific ventral wing coloration because, when at rest or when feeding, it deters approaches of mate-seeking males and thus avoids costs in time and energy spent on assessing sexual identity (Sherrat & Forbes, 2001).

Summary

Poulton (1890) suggested that senders and receivers of iridescent signals should orient themselves in the environment such that signal transmission and reception are maximized. To evaluate this hypothesis, details of how an iridescent signal's characteristics vary with signaller, receiver, and light source positions must be determined. Moreover, this determination should incorporate the colour vision of the interactants and should be framed in an ecologically relevant context. In the present study, we have estimated how the UV iridescent signal of male *C. eurytheme* appears to conspecifics under natural lighting and against the primary visual background (alfalfa) where they occur in our geographical region (central Arizona, USA). The results of our analyses indicate that optima exist for sender and receiver positions: the UV signal is viewed at maximum brightness and for the longest duration when the receiver is positioned directly above the signaller. Our results also suggest that differences in male and female wing coloration should be discerned readily by the butterfly visual system under typical ambient illumination, and that males are more conspicuous than females against typical backgrounds. Based on the premise that the male UV signal is valuable to females in mate choice, we make the prediction that courting males should position themselves directly below females to maximize signal reception, regardless of the orientation of the sun. We plan to test this prediction under field conditions in future research.

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